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(54) Method for carrying out immunofluorescent assays.

(57) Method for carrying out immunoassays, in which method an antibody (or antigen) marked with a tracer so as to be fluorescent is attached onto an antigen (or antibody, respectively) present at the inside wall of the measurement vessel, a liquid denser than the sample is added to the measurement vessel, which said denser liquid displaces the liquid from underneath, and both the excitation radiation is passed into the sample and the fluorescent radiation is collected to the detector through the wall or the bottom of the measurement vessel.

Method for carrying out immunoassays

The present invention is concerned with a fluorometric or phosphorimetric immunoassay method in which an antibody or antigen marked with a fluorescent or phosphorescent tracer is attached to the inside wall of the measurement vessel.

In fluorometric immunoassays (FIA), the antigen or antibody has been adsorbed, e.g., onto cuvettes or microtiter discs made of polystyrene or polyacrylics. This so-called antigen or antibody bound to the solid phase is allowed to react with the antibody or antigen, respectively, present in the sample to be studied and with the antibody or antigen, respectively, marked with a fluorescent molecule. The less antibody or antigen there is present in the sample, the more of the marked substance adheres to the solid phase. When the marked antibody or antigen remaining in the liquid phase is separated, and when the quantity of the marked substance adhering to the solid phase is measured by means of a fluorometer, the concentration of the antibody or antigen in the sample is found out.

In the prior-art methods, any excessive marked antibody or antigen must be washed off before the fluorometric measurement.

The object of the present invention is to provide a fluorometric solid-phase immunoassay method in which the excess tracer does not have to be removed out of the measurement vessel before the fluorometric measurement.

In the method in accordance with the invention, the antigen or antibody is attached to the wall or bottom of the measurement vessel, and the fluorescent radiation is measured through the wall or bottom. After the reaction of the sample and of the added, marked antibody or antigen with the antigen or antibody, respectively, of the solid phase has taken place,

a liquid denser than the sample is added into the measurement vessel, which said denser liquid displaces the sample from underneath,

In the method in accordance with the present invention, emptying and washing of the measurement vessel can be omitted. In this way, all the transfers of liquid related to the assay are additions of liquid. This makes the assay more rapid and faciliates its automatization decisively.

When a liquid denser than the sample is added to the sample in the method, the liquid is most appropriately coloured so that it has a strong absorption of light either at the excitation wavelength of the fluorescence or at the emission wavelength, or at both, being thus, e.g., of black colour. In this way, the fluorescence can also be measured straight from below without interference by the excess tracer or by the background radiation. Thus, the method is preferably accomplished so that the solid phase is placed on the bottom of the measurement vessel and that both the excitation light is passed into the measurement vessel and the fluorescent light is collected to the detector through the bottom of the measurement vessel.

In the method, the liquid and colouring agent added must, of course, be such that they do not have an effect disturbing the measurement on the reaction between the antigen and antibody and that they do not prevent fluorescence of the tracer.

The colouring, if any, must be so strong that the fluorescence of the tracer present in the free liquid of the sample is not seen to a significant extent in the measurement channel. If the coloured liquid is mixed with the sample, the absorption coefficient must be very high. If the coloured liquid does not mix with the sample and is heavier than the sample, an absorption factor of an order of 1/mm is sufficient if the thickness of the coloured liquid layer is several millimetres.

Well suitable for a non-miscible liquid denser than water are, e.g., such fluorinated hydrocarbons as are liquid at the room temperature, such as, e.g., trichlorotrifluoroethane (Freon - 113) and dibromotetrafluoroethane (Freon - 114B2). These can be coloured black, e.g., by means of fine carbon powder (soot).

According to one embodiment, a liquid denser than water is added into the measurement vessel, which said liquid is allowed to solidify before measurement.

Of course, the method can also be applied to a method in which phosphorescence is made use of.

WHAT IS CLAIMED IS:

1. Method for carrying out immunoassays, in which method an antibody or antigen marked with a fluorescent or phosphorescent tracer and attached to the bottom or to the side walls of the measurement vessel is excited by means of appropriate radiation and the fluorescent or phosphorescent radiation emitted from the antibody or antigen is measured, c h a r a c - t e r i z e d in that, before the measurement, a liquid denser than the sample is dosed into the measurement vessel, which said denser liquid displaces the liquid from underneath, and

that both the excitation radiation is passed into the sample and the fluorescent or phosphorescent radiation is collected to the detectors through the wall or the bottom of the measurement vessel.

2. Method as claimed in claim 1, c h a r - a c t e r i z e d in that a liquid denser than the sample is dosed into the measurement vessel, which said denser liquid absorbs strongly at the wavelength of the excitation radiation.

3. Method as claimed in claim 1 or 2, c h a r a c t e r i z e d in that a liquid denser than the sample is dosed into the measurement vessel, which said denser liquid absorbs strongly at the wavelength of the emission radiation.

4. Method as claimed in claim 2 or 3, c h a r a c t e r i z e d in that the liquid denser than the sample is black.

5. Method as claimed in any of claims 2 to 4, c h a r a c t e r i z e d in that both the excitation radiation is passed into the sample and the fluorescent or phosphorescent radiation is collected to the

detector through the bottom of the measurement vessel.

6. Method as claimed in any of the claims 1 to 5, c h a r a c t e r i z e d in that a liquid denser than the sample is dosed into the measurement vessel, which said denser liquid is allowed to solidify in the measurement vessel.